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Determination of Flavonoid and Caffeine Content in Black and Oolong Teas

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Abstract: The world is blessed with tea for its health benefits and the hundreds of flavours to be chosen from. Flavonoid and caffeine is the most abundant type of phenolic compound and alkaloid found in tea respectively. These compounds can be extracted using various solvents, resulting in various concentration when compared with water as the solvent. This study was conducted by preparing tea infusion from both distilled water and organic solvents to observe the effect of solvents on the content of flavonoid and caffeine. Using spectrophotometric method, the content of flavonoid and caffeine in commercial black (Boh, Sabah, Lipton) and oolong (Oo Long Kee Zhong, Montea, Ten Ren) teas obtained from Malaysian market were determined. Sabah and Montea has the highest flavonoid (0.044 g/L) and caffeine (3.585 g/L) content in distilled water respectively. The final content of flavonoid and caffeine was found to be higher in organic solvents compared to distilled water, as well as when the steeping time is increased.

Keyword: Tea, flavonoid, caffeine, spectrophotometric.

1. Introduction

It is no surprise to find various types of tea being offered in stores and restaurants in almost every parts of the world. Tea is the second most consumed beverage in the world, after water, with an estimate of 20 billion cups enjoyed every day [1–4]. To make tea drinks, the leaves of *Camellia sinensis* plant is used, and the variety of types and flavours of teas are obtained from different processing method. There are two major cultivars of this plant which are var. *sinensis* that is mainly grown in China and Japan, and var. *assamica* that is dominant in South and South East Asia [4–6]. Chinese tea (var. *sinensis*) has smaller leaves of about 5 – 12 cm while Indian tea (var. *assamica*) has leaves that may reach length of 20 cm [5,7]. Tea was first cultivated in China, practised as traditional medicine before being acknowledged as daily beverage across the globe [1,4,8,9].

Three main types of tea are green tea, black tea and oolong tea. These teas are characterized by the degree of fermentation where fermentation is the process of oxidising green tea to produce black tea [3,10]. Enzyme polyphenol oxidase is present in fresh tea leaves which is activated when the leaves are cut, crushed, or rolled. It is inactivated when the leaves are heated [11,12]. In fermentation, chemical constituents in tea leaves undergo enzymatic conversion or being oxidised by the enzyme [4]. Green tea is made from non-fermented tea leaves, therefore it is only rolled and heated to prevent oxidization. To make black tea, the leaves are rolled, then fermented to allow oxidization of phenolic compounds in the tea, and then heated [11–13]. Oolong tea is the intermediate between green tea and black tea, therefore it is made of partially fermented leaves obtained from shorter fermentation period [6,14].

Drinking tea not only quenches thirst but also brings health benefits. While black tea may decrease blood pressure; green tea may regulate body temperature, blood sugar and enhance digestion; and oolong tea is excellent for its anti-diabetic, anti-obese and anti-inflammatory properties [15]. Tea being a beverage packed with goodness is contributed by its myriad chemical constituents. The complex chemical composition of tea includes phenolic compounds, alkaloids, amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, minerals, and trace elements [16,17].

Flavonoid and caffeine is the most abundant type of phenolic compound and alkaloid found in tea respectively [4,18]. Phenolic compounds is considered the most important constituent of tea since it is the largest component and act as bioactive ingredient that enhances the therapeutic action

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of tea [4,15]. Caffeine, in the other hand, provides both benefit and disadvantage because it stimulates the central nervous system [11,19].

In this study, the content of flavonoid and caffeine for commercial black and oolong teas in Malaysian market are determined using spectrophotometric method. Black and oolong teas were chosen as to observe if there is any significant difference in the content which may be caused from fermentation. Data from previous researches showed that different concentration were obtained when different solvents were used. When organic solvents are used, the consumers cannot be sure of the real content may present in their cup of tea because they are using only water to make their cuppa. This thus motivates the designation of this study to view the effect of distilled water and organic solvents in the final content of flavonoid and caffeine. The effect of steeping time in influencing the final content is also observed. The data provided may also guide Malaysian consumers in choosing the type of tea that best suits their preferences.

2. Materials and Method

Samples. A total of six commercial teas were purchased from Malaysian supermarket consisted of three black teas (Boh, Sabah, Lipton) and three oolong teas (Oo Long Kee Zhong, Montea, Ten Ren). The teas were removed from the tea bags and homogenized for further analysis.

Chemicals and instrumentation. Chloroform (99%, QRec), hydrochloric acid (37%,QRec),sodium nitrite (37%, QRec Asia), sodium bicarbonate (99.5%, Dulab), methanol (99.98%, HmbG Chemicals), sodium hydroxide (98%, Prolabo), ferric chloride (98%, Emory), aluminium chloride (99%, Emory), sulfuric acid (95%, Emory), nitric acid (65%, Friendemann Schmidt), potassium thiocyanate (98.5%, Bendosen), analytical balance (RADWAG, WAS220, 10mg – 220g), UV-Vis spectrophotometer (PG Instruments, T60, 325 – 1100nm), centrifuge (MPW Med. Instruments, MPW-350R, 450 – 18,000 rpm).

Flavonoid content determination. The flavonoid content in different tea samples were determined using quercetin as the standard [6,7,20,21]. Quercetin standard solutions were prepared using different solvents (distilled water and methanol) to produce the calibration curve for flavonoid content determination. 12, 24, 36, 47, and 59 mM of quercetin were diluted for distilled water infusion and methanol infusion. 10 mL of water infusion at time interval 5, 10, 15, 20, 25, and 30 minutes and methanol infusion (1, 2, 3, 4, 5, and 24 hours) were withdrawn and cooled to room temperature. After centrifugation, 0.3 mL distilled water and 0.03 mL sodium nitrite were added into the solutions. The solutions were left for 5 minutes at 25 ºC, and 0.03 mL aluminium chloride was added. The solutions were left for another 5 minutes followed by the addition of 0.2 mL 1 mM sodium hydroxide and 1 mL distilled water. The absorbance for the prepared solutions were measured using UV-Vis at 510 nm. For the measurement of flavonoid in tea samples, the caffeine was replaced with 0.500 g of tea leaves. Flavonoid content was expressed as mg of quercetin equivalent per gram of sample (mg QE/g).

Caffeine content determination. Different concentrations of caffeine standard solutions (0.4, 1.0, 1.4, 1.8, and 2.4 g/L) were prepared in different solvents; distilled water and chloroform.. The absorbance was measured at 272.8 nm and 276 nm for water infusion and chloroform infusion respectively. To measure caffeine content in tea samples, 25.000 g of tea leaves was heated in 300 mL distilled water at 100 ºC. At 5 minutes interval for 25 minutes, 20 mL of the solution was withdrawn and the absorbance was measured at 272.8 nm. 50 mL of chloroform was added to the withdrawn samples and the solutions were stirred continuously for 10 minutes. The chloroform phase separated was then analysed with UV-Vis at 276 nm.

3. Results and Discussion

Flavonoid content. Of various phenolic compounds present in tea, flavonoid is the most abundant. Flavonols or catechin, a class of flavonoid, is the major constituent in fresh tea leaves, accounting for up to 30 – 42 % of dry leaf weight [5,11,13,18]. Phenolic compounds available in tea leaves are responsible for the antioxidant activity of tea that can promote good health by scavenging free radicals harming the body [4,6,22].

The flavonoid content obtained from water and methanol infusion in different extraction time according to the type of solvent is as summarized in Figure 1, 2 and 3. Flavonoid can

be tested in various solvent such as water, methanol, ethanol and acetonitrile [23]. Methanol used to determine the total flavonoid in tea infusion from the previous research found that methanol gave higher flavonoid content as compared to hexane [24].

In Figure 1, the content of flavonoid in the tea samples by water infusion method is shown. The flavonoid content can be seen increasing from 0 minute to the 30th minute. Black tea samples showed higher flavonoid contents compared to oolong teas. Within 30 minutes, Sabah black tea recorded the highest flavonoid content of 8.8 mg QE/g while the rest of tea samples have flavonoid content of 8.6 mg QE/g. In 2014 Pereira *et al.*, measured the flavonoid content of Brazilian black teas using the same spectrophotometric method and recorded average values of 6.11 – 8.75 mg QE/g flavonoid in the samples[7]. The usual value of non-oxidised catechin in black and oolong teas are 10 % and 8 – 20 % respectively [5]. The value obtained for tea samples in this study satisfy the mentioned value, however, indistinct values of flavonoid between black and tea samples may be due to the short period of extraction time.

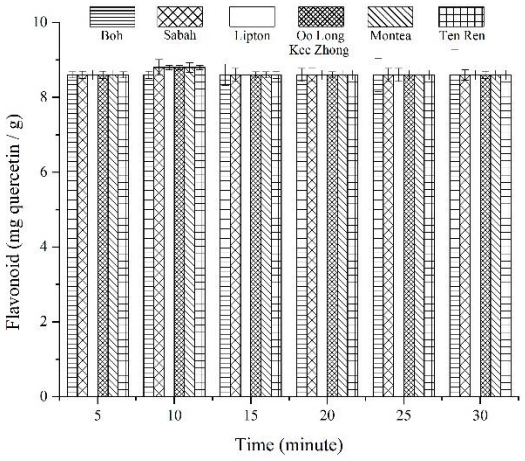

Fig. 1 Flavonoid content of tea samples by water infusion for 30 minutes

The bar charts in Figure 2 and 3 showed the flavonoid contents in the tea samples using methanol infusion method. From methanol infusion, it can be seen that black tea samples showed higher flavonoid contents compared to oolong teas. At the first hour, the content of flavonoid in all tea samples was higher than the previous 30 minutes flavonoid content recorded through water infusion. The highest flavonoid content at 1 hour extraction time was recorded for Sabah black tea (0.058 g/L) followed by Boh black tea (0.055 g/L), Lipton (0.054 g/L), both Montea and Ten Ren oolong tea (0.053 g/L), and the lowest is Oo Long Kee Zhong (0.052 g/L). At the following hours, the flavonoid contents increases steadily for all tea samples.

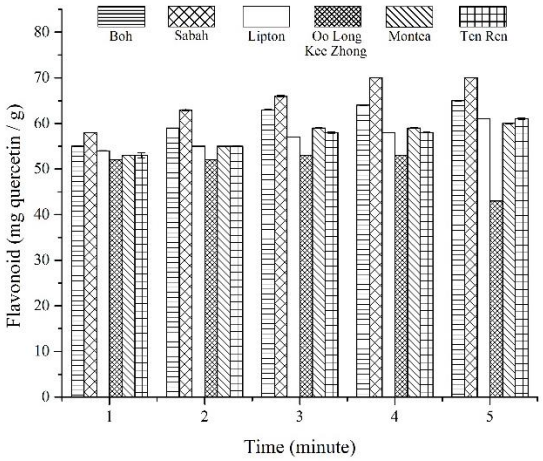

Fig. 2 Flavonoid content of tea samples by methanol infusion for 5 hours

At 24 hour, Sabah black tea still recorded the highest flavonoid content of 0.078 g/L followed by Ten Ren oolong tea (0.076 g/L), Boh black tea (0.073 g/L), Lipton black tea (0.071 g/L), Montea oolong tea (0.069 g/L), and lastly, Oo Long Kee Zhog oolong tea (0.057 g/L).

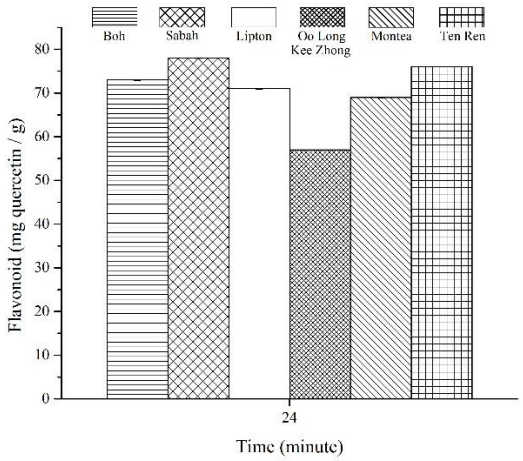

Fig. 3 Flavonoid content of tea samples by methanol infusion at 24 hours

Generally, it can be seen that extraction time also plays an important role in determining the flavonoid content of tea leaves. Rusak *et al.*, confirmed in their study with related to studies done by previous researches that the content of polyphenols such as flavonoid reaches the

maximum at 30 minutes extraction time [13]. However, in water infusion with extraction time of only 30 minutes, the flavonoid content is significantly lower than the flavonoid contents of the same samples using methanol 24 hour extraction. Black tea samples in this study showed greater flavonoid content than oolong tea samples. Hertog *et al.*, (1993) discovered that the flavonoid contents of selected types of flavonoids for oolong tea is generally in the lower range of those in black tea [25].

Non-fermented teas (green tea) contain simple flavonoids (catechin) which oxidise to complex flavonoids (theaflavins and thearubigens) by the action of enzyme [4,6,10,13]. Figure 4 and 5 illustrate the structure of catechin and theaflavins. Oxidisation of catechin to theflavins during the fermentation process gives the dark colour and stringent flavour to the tea [1,5,12].

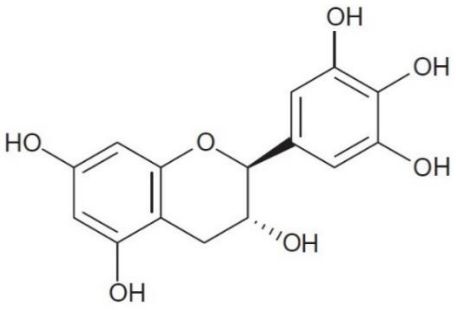

Fig. 4 Structure of catechin

The content of flavonoid may contribute to antioxidant activity in teas that reduces the risk of chronic disease by scavenging free radicals [6,14,25]. Although fermented teas may contain only little amount of catechin compared to non-fermented teas, the conversion of catechin to theaflavins however does not affect the antioxidant activity of black teas [16,26].

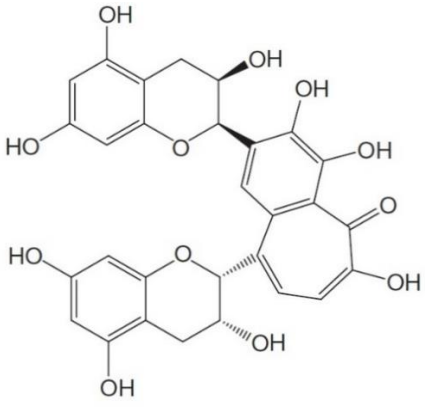

Fig. 5 Structure of theaflavins

Free radicals can alter cell membrane properties, break DNA bonds, damage cell's genetic apparatus and eventually cause cancer [22]. Antioxidant can prevent the notorious effect of free radicals because of the phenolic hydroxyl group attached to the flavan-3-ol structure [16,22]. Antioxidant activity is linked to the flavonoid content where the higher content of flavonoid correlates to higher number of antioxidant [7,22,27]. Thus it can be interpreted that black tea samples in this study may contribute to higher antioxidant activity then oolong tea samples.

Caffeine content. Caffeine is the most abundant alkaloid, or nitrogen-containing substance in tea and it gives the bitter taste of tea [4,28]. The structure of caffeine is shown in Figure 6. Known as central nervous system stimulant, caffeine improves alertness and relaxes the muscles [28–30]. Though the content of caffeine in tea is not as high as those in coffee, but caffeine is also known to induce anxiety, panic attacks, headache, and sleeplessness if caffeine is taken too much per day (exceeding 200mg/day) [11,29].

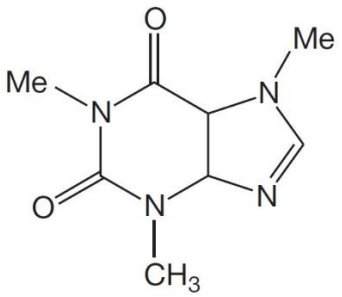

Fig. 6 Structure of caffeine (Me represents methyl group)

Caffeine can be extracted from plants such as tea using water and various organic solvents such as chloroform [28]. Water and chloroform are used as solvents to determine the infusion of caffeine and the caffeine content in the tea samples. Both of solvents are not flammable and not too dangerous to handle. The Chloroform was chosen because the solubility of caffeine in chloroform is higher than other organic solvents such as dichloromethane, acetone and ethyl acetate [28,31].

Steeping time is considered as one of the factor that influences caffeine content in a solvent. Steeping time is the duration of time used to allow flavour from tea to be extracted right after the tea leaves were placed in water [29].

The extraction of caffeine in distilled water and chloroform within 25 minutes are shown in Figure 7a and 7b. The infusion of caffeine in chloroform is higher than distilled water. As reported by Yang *et al.* (2007) and Raghuwanshi *et al*. (2014), the content of caffeine extracted is increasing with increased steeping time [19,29]. This trend is also presented in Figure 7a and 7b.

In both solvents, Montea showed greater caffeine extraction through time, indicating that its caffeine content is higher than the other tea samples. The caffeine content of the studied tea samples at minute 25th is as summarized in Table 1. The highest content of caffeine was given by Montea oolong tea of 3.59 g/L in distilled water, and 3.88 g/L in chloroform. In water infusion, the lowest content of caffeine was 3.46 g/L by Lipton black tea whilst for chloroform infusion, Lipton and Ten Ren oolong tea recorded the same content of 3.86 g/L caffeine. Although the same steeping time of 25 minutes was used, the caffeine content is higher in chloroform because chloroform is a better solvent in extracting caffeine than water as reported by previous studies [28,32].

It has to be taken into considerations that consumers make their tea using water, therefore, the caffeine value extracted from distilled water is the closest representative of how much caffeine is released in household tea, and the caffeine content obtained from chloroform may indicate the more exact amount of caffeine present in the tea sample.

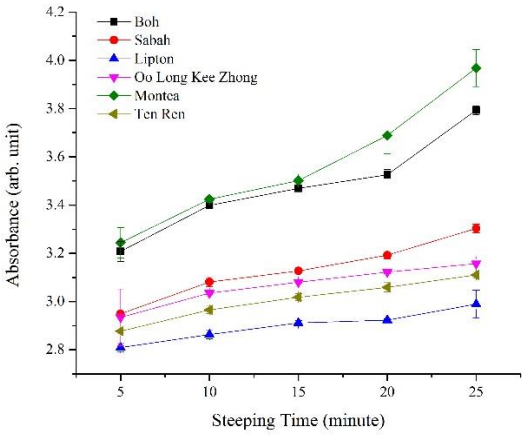

Fig. 7a Water infusion of caffeine at 25 minutes steeping time

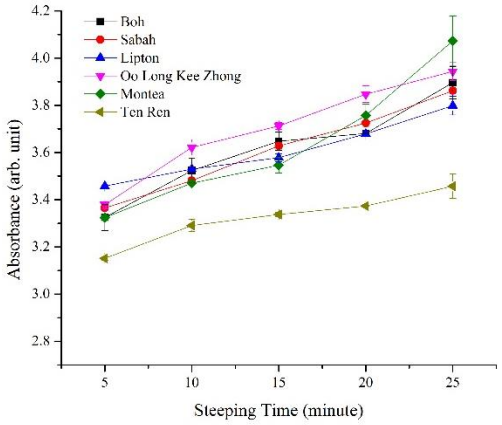

Fig. 7b Chloroform infusion of caffeine at 25 minutes steeping time

Table 1 Caffeine content in tea samples extracted in distilled water and chloroform in various steeping time.

Sample	Caffeine content in different solvents (g/L)									
	Distilled water					Chloroform				
	5 min	10 min	15 min	20 min	25 min	5 min	10 min	15 min	20 min	25 min
Boh	3.49	3.51	3.52	3.53	3.56	3.85	3.85	3.86	3.86	3.87
Sabah	3.45	3.47	3.48	3.48	3.50	3.85	3.85	3.86	3.86	3.87
Lipton	3.43	3.44	3.45	3.45	3.46	3.85	3.86	3.86	3.86	3.86
Oo Long Kee Zhong	3.45	3.46	3.47	3.48	3.48	3.85	3.86	3.86	3.87	3.88
Montea	3.49	3.51	3.52	3.55	3.59	3.84	3.85	3.86	3.87	3.88
Ten Ren	3.44	3.45	3.46	3.47	3.47	3.84	3.84	3.85	3.85	3.86

For consumers who prefer tea with less caffeine content, Lipton black tea is the best choice, and Montea oolong tea is suggested for those who are not.

4. Conclusion

The content of flavonoid and caffeine determined by spectrophotometric method revealed that higher concentration was obtained

when organic solvent is used compared to distilled water. The steeping time also pose an influence where the longer the steeping time, the higher the final content recorded.

Acknowledgements

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